Cytomegalovirus:

Clinical Virological Correlations in Renal Transplant Recipients

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One-hundred thirty-two renal transplant recipients were systematically screened for viral infections and the findings correlated with the clinical course. One-hundred ten patients showed evidence of infection with herpesviruses and 89 patients showed laboratory evidence of infection with cytomegalovirus (CMV) uncomplicated by bacterial infections or technical complications. Patients without viral infections were usually asymptomatic. After recovery and development of anti-viral antibodies, most patients were asymptomatic despite the persistence of viral excretion in the urine. In contrast, the onset of viral infections were almost always accompanied by a significant clinical illness characterized by fever, leukopenia, and renal malfunction. Of 89 patients with cytomegalovirus infections, 83 survived at least three months. In these patients, the fever appeared to be self-limited and resolution of the fever was accompanied by increases in anti-CMV antibody. Renal biopsies demonstrated typical rejection reactions in all the biopsied patients and renal malfunction usually responded to anti-rejection treatment. Six of the 89 patients with CMV infections died within a month of viral isolation. These patients could be distinguished from those who recovered by a decreased or absent antibody response to the virus, suppressed lymphocyte responses to mitogen in autochthonous blood, and absent histologic evidence of rejection in the renal allografts. Thus, two paradoxical responses to CMV infections are seen in transplant patients: In the relatively immunocompetent patient, the infection is associated with renal allograft rejection, a prompt antibody response to the virus, and recovery. The severely immunosuppressed patient cannot make an antibody response, does not exhibit allograft rejection as a cause of renal malfunction, he may be further immunosuppressed by the viral infection, and is susceptible to sequential opportunistic infections leading to death.

Hill *et al.*,[17] and Hedley-Whyte and Craighead,[16] were the first to implicate cytomegalovirus (CMV) in the pathogenesis of clinical disease in allograft recipients, when they found CMV inclusion cells in the lung and other tissues of patients who died of pneumonia after transplantation. This relationship has been frequently confirmed and in some surveys, including our previous study, infection rates of 70–90% have been established.[2,8,30] The common finding of CMV in asymptomatic patients after transplantation has suggested that CMV may be an incidental finding in such patients. On the other hand, CMV infections have been implicated in the triggering of rejection episodes,[7,30,48] in the pathogenesis of mild febrile illnesses,[9,11] hepatitis,[3,11,12] pneumonia[16,42,47] and even in the pathogenesis of death. This study represents part of a continuing[29–31] effort to critically analyze the role of these latent viral infections in transplant patients, and to correlate the viral infections with the clinical picture.

Materials and Methods

The procedures for the selection of donors and recipients, techniques of transplantation, standard immunosuppressive regimens, and diagnosis and standard treatment for rejection episodes have previously been described.[46] Briefly, the immunosuppressive regimen in-

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cluded methylprednisolone (20 mg/k) on the day of, and the two days after transplantation. Antilymphocyte globulin (ALG) (20–30 mg/kg) for 14 days after surgery, and decreasing amounts of azathioprine and prednisone to maintenance doses (Figs. 2, 4). The study group consisted of 132 patients who had received renal allografts at the University of Minnesota Hospitals. 113 of these patients were studied after transplantation and 19 patients were chosen at random before the transplant for serial study through the pretransplant dialysis and posttransplant course.

Viral studies were carried out either at the clinical virology laboratory of the University of Minnesota or in the virus laboratory of the Minnesota Department of Health. Fresh urine, sputum, blood, and stool samples were collected for viral isolation weekly for two to six weeks after the patient was placed on the study, and then once every two to four weeks thereafter. Serum was collected for antibody determinations at the same intervals. The methods of specimen collection, processing, and inoculation were as previously described.[5,30] Viral isolates were identified by neutralization according to the usual methods[28] with antisera provided by the National Center for Disease Control, Atlanta, Georgia. CMV isolates were identified by: 1) characteristic cytopathic effect; 2) growth only in human embryonic diploid fibroblast cell cultures; 3) complement fixation (CF) using the patient's isolate as antigen and human convalescent CMV antisera known to be negative for CF antibodies to *Herpesvirus hominis* and varicella-zoster viruses, also supplied by the National Center for Disease Control; and 4) indirect immunofluorescence using convalescent human CMV antisera.

Complement fixing CMV antibody titers were performed in a microtiter system according to standard methods[28] using the AD-169 strain of CMV obtained from Microbiological Associates, Bethesda, Maryland as antigen. The antigen for the varicella-zoster complement fixation test was also obtained from Microbiologic Associates, and the *Herpesvirus hominis* type 1 CF antigen was obtained from Flow Laboratories, Rockville, Maryland.

A significant febrile episode was defined as a temperature of 101 F orally for two or more days within a seven-day period. Leukopenia was defined as a total peripheral white cell count less than 5,000 cells/mm^3 for a period of at least two days. Clinical laboratory evidence of rejection was defined as an increase in the serum creatinine level of at least 0.5 mg/100 ml. The patient was considered to have a viral infection if a virus was isolated from any cultured material or if the patient demonstrated a fourfold or greater rise in any of the viral antibody titers measured.

Serial immunologic studies were performed on 61 of the 132 patients. Serum and heparinized blood were collected at weekly intervals during the two to four weeks after the patients were started on the study and then once every four to eight weeks thereafter. Cell-mediated immunity was assayed by *in vitro* lymphocytic responses to phytohemagglutinin (PHA) using the micro-PHA test described by Park and Good[35] and a commonly used macro-PHA test.[15] The micro-PHA test utilizes heparinized whole blood and gives a quantitative estimate of the patient's thymus dependent cell (T-cell) function in peripheral blood. Since the test is conducted in the patient's own serum, lymphocytic responses, as well as serum factors which may affect their responses, contribute to the final result. The macro-PHA test was carried out with slight modifications of a previously described method.[15,31]

Results

Herpesvirus Isolation and Seroconversion

Although the virologic methods used in this study are capable of isolating many viruses, only members of the herpesvirus group, namely, cytomegalovirus (CMV), herpes zoster virus (HZV), and herpes simplex virus (HSV) were consistently isolated. A poliovirus was isolated from each of two patients but these isolations could not be repeated one week later and clinical manifestations were not associated with the isolation. Parainfluenza and adenovirus I were also isolated on single occasions and were not associated with clinical syndromes. Several patients had hepatitis associated antigen in their serum, but as others[40,50] have observed this is not usually associated with clinical symptoms or alterations in liver function in transplant patients.

Table 1 details the herpesvirus infections detected in the study group. Of the 132 patients studied, 110 (83.5%) had laboratory evidence of at least one infection with a herpesvirus whereas the remaining 22 (16.7%) had no evidence of a virus infection. Since 113 of 132

TABLE 1. *Cytomegalovirus and Other Herpesvirus Infections in 132 Renal Transplant Recipients*

No Infections (22)		Herpesvirus Infections (110)	
Early transplant failures	6	Cytomegaloviruses*	
No infections	16	Time of infection obscure	43
		Onset of infection detected	46
		Other complications	10
			99
		Other herpesviruses	11
Totals	22		110

* Twenty-three of these patients had cytomegalovirus infections plus other herpesvirus infections, 18 had cytomegalovirus plus herpes hominis, 3 had cytomegalovirus plus varicella zoster, and 2 had cytomegalovirus, herpes simplex virus and herpes zoster infections.

patients were admitted to the study because of clinical symptoms of various types, a truer incidence of virus infections was determined using the group of 19 patients selected at random before the transplant and studied at intervals before and after transplantation. Sixteen (84.5%) of these 19 had at least one infection with a herpesvirus; 14/19 (74%) had a CMV infection, 4/19 (21%) had a HSV infection, and 1/19 (5.3%) a HZV infection. The incidence of herpesvirus infections in the small group of patients chosen at random is surprisingly similar to the larger group of selected patients (Table 1).

Twenty-three of the 132 study patients had infections with two or more herpesviruses; 18 patients had CMV and HSV infections, three had CMV and HSV infections and two had CMV, HSV, and HZV infections (Table 1).

Cytomegalovirus Isolation or Seroconversion

The remainder of the results will focus only on those patients with cytomegalovirus. Diagnosis of CMV infections was based on the isolation of virus in 72/99 cases (10/14 in the random cases). The diagnosis was based on serological evidence alone in 26 of the 99 cases (4/14 in the random cases). Sixty-six of the 72 cases in whom virus was isolated showed seroconversion to CMV by a fourfold increase in complement fixation titer. CMV was isolated from the urine alone in 23 patients, from the sputum (or from bronchoscopic washings) alone in 13 patients, from the urine and sputum in 33 patients, and from biopsy or autopsy tissue alone in 3/72 patients in whom virus was isolated. CMV was, therefore detected in the urine of 56 of the 72 patients in whom virus was isolated. Of the 56 patients who showed viruria, 44 (78.5%) continued to excrete virus in their urine for at least five months after seroconversion and at least one patient for 12 months. Reappearance of viruria was seen one or more years posttransplant in several patients with high (>1/256) serum titers of complement fixing antibody.

Clinical Manifestations of Cytomegalovirus Infections

Uninfected Patients. Only 22 of the 132 study patients showed no laboratory evidence of viral infections. Six of these 22 patients either had technical complications or were not studied long enough to be included in this analysis. Of the 16 remaining patients, three were among the 19 selected at random who were studied serially before and after they received their transplant. Eight of the 16 patients have remained free of detectable virus infections for six consecutive months after receiving the transplant and two have remained virus free for 20 consecutive months. Only 7/16 virus-free patients had a rejection episode and only 1/16 had the syndrome of fever, leukopenia, and clinical rejection.

CMV Infected Patients. Ninety-nine patients had laboratory evidence of CMV infections. The clinical-virological correlations were obscured in ten patients who had obvious bacterial or technical complications; these ten patients have, therefore, been excluded from the following discussion of clinical correlation. In addition, the time of onset of viral infections could not be determined in 43 of 99 patients with CMV infections who were found to have complement fixing (CF) antibody titers $\geq$16 when first studied (28/43 patients whose CF antibody titers were high when first studied were also excreting CMV). Although fourfold or greater increases in CF antibody titers were detected in these 43 patients the exact time of onset of seroconversion could not be determined since the titers were already rising when first studied. Clinical correlations in these patients will be discussed below.

Forty-six of the 99 patients in whom CMV infections developed were studied early enough and thoroughly enough to accurately determine the time of onset of their infections. Onset of virus infections was defined as a positive culture after several (at least three) attempts had been negative, or a fourfold or greater increase in CF antibody titer when the initial titer was $\leq$8. Negative cultures were not detected prior to the positive cultures found in 10 patients. In each of these patients, seroconversion alone was used to determine the time of onset of virus infections. In six patients, serum antibodies to CMV never developed and onset of infection in these patients was determined by the first positive culture after several attempts had been negative. In 45 of these 46 well-studied patients, clinical findings could be correlated with the onset of virus infections, whereas in only one case the initial isolation of virus or seroconversion was not associated with any clinical illness. Eleven of these 46 patients were among the 19 patients selected at random.

Thirty-three of these 46 patients (14 of the 15 randomly selected patients) with laboratory evidence of CMV infections had fever (defined as an oral temperature greater than 101 F for 2 days in any 7-day period) whereas only four patients without a virus infection had such febrile episodes after receiving a transplant. The average time after fever for seroconversion was 29.7 days. The average time after fever and before the first positive CMV culture was 18 days. Each patient was his own control since 27 of the 33 patients were afebrile during the period after transplantation and before the febrile episode later associated with the virus infection.

Only two patients had positive viral cultures three and 21 days before the febrile episode. Positive cultures

were collected three to 11 days after the onset of fever in only 8/31 patients from whom virus was isolated. Although CMV could sometimes be isolated at the time of onset of fever or shortly thereafter, the average time of viral isolation was only four days before seroconversion. Since a rise in CF antibody cannot normally be detected until 14 to 28 days after clinical infections,[10,23] seroconversion appears to be a better indicator of the onset of virus infection than isolation of the virus.

CMV infection was also shown to be associated with episodes of leukopenia (defined as a total peripheral leukocyte count less than 5000/mm^3 for two or more consecutive days). Thirty of the 46 patients (8/15 randomly selected patients) with laboratory evidence of CMV infections developed leukopenia; whereas only one of the patients without virus infections was leukopenic after transplantation. Thus, of 31 cases with leukopenia, 30 had laboratory evidence of CMV infection. Onset of leukopenia preceded seroconversion by an average of 30.0 days. In seven patients a rise in antibody titer preceded leukopenia by 4, 6, 7, 28, 57, 60, and 118 days. The average time from onset of leukopenia to positive culture was 12.1 days. Leukopenia followed the first positive culture in five cases by 2 and 12 days and in one case by 132 days. Many of these

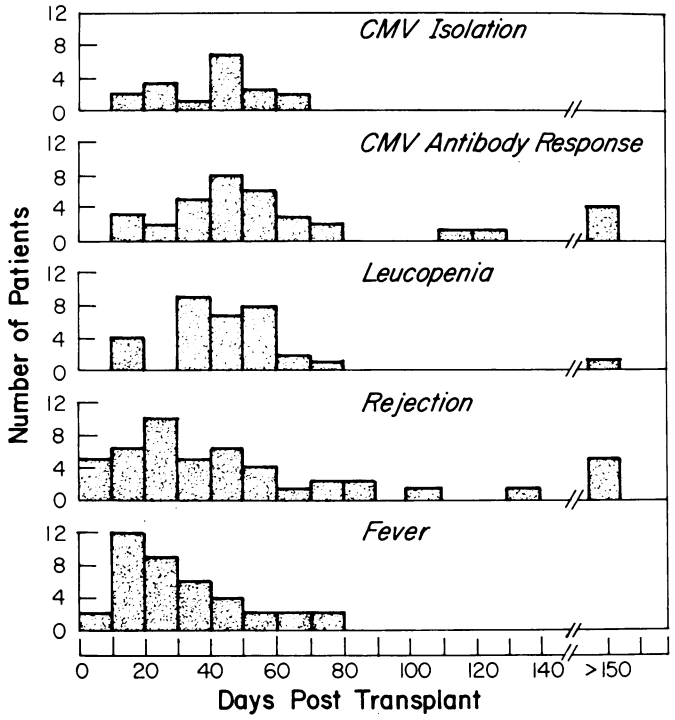

FIG. 1. Composite clinical course of 46 renal transplant patients with known time of onset of cytomegalovirus (CMV). Fever, renal malfunction and leukopenia are followed by the development of antibodies to CMV. Viral isolation is not always possible until seroconversion occurs.

patients had several leukopenic episodes after the onset of the virus infection and while they continued to have high antibody titers and to excrete virus. However, no other episodes of leukopenia were detected in these patients between the date of transplantation and the onset of CMV associated leukopenia.

An association between CMV infections and episodes of elevated serum creatinine was also evident. Thirty-seven of the 46 patients (7/15 randomly selected patients) with laboratory evidence of CMV infections had an episode of clinical "rejection" whereas only 7/16 control patients without virus infections showed signs of rejection. Thus, of 44 patients with rejections, 37 had CMV infections. Only one episode of allograft rejection occurred during the period of time after transplantation and prior to the virus associated rejection episode. The average time after rejection for seroconversion was 20.0 days. One patient had his first rejection episode 107 days after the onset of the virus infection and long after his antibody titer had become elevated. CMV was isolated from 32 of the 37 patients, an average of 26.6 days, after rejection. Of the 32 patients virus was isolated *after* the onset of rejection in 19. In six patients the virus was isolated during the first 3 days of the rejection episode and in 7 others the virus was isolated 162, 121, 24, 18, 12, 3, and 2 days before the onset of rejection. Thus, in only 13 patients could it be said that the *virus appeared* prior to or simultaneously with a rejection episode.

Of the 46 patients with known time of onset of CMV infections, 5 developed fevers without clinical rejections, 8 had clinical rejections without febrile episodes, 29 developed both fevers and clinical rejection, and only 4 had neither clinical illness associated with their virus infections. Whether the rejection episode occurred in conjunction with a febrile episode or not, the fever was always closely correlated with laboratory evidence of virus infection. Of the 29 patients who developed both fevers and rejection in association with CMV infection, 22 (76%) developed their initial fevers before or on the same day as the rejection episodes.

The typical syndrome can be seen by examining the frequency distribution of times after transplantation that fever, leukopenia, elevated serum creatinine, and laboratory evidence of CMV infection appeared (Fig. 1). Laboratory diagnosis of CMV infections were made in highest incidence between 30 and 70 days after transplantation, and shortly after the peak incidence of fevers, leukopenia and renal functional deterioration.

The clinical course of a typical patient with this syndrome is shown in Fig. 2. Spiking fevers were followed by renal functional deterioration and leukopenia. CMV infection was demonstrated by a rise in CF antibody titer within 2 weeks and virus was isolated from urine.

Biopsy of the donor kidney at the time of elevated serum creatinine revealed histological changes consistent with classical rejection (Fig. 3). Treatment with increased doses of steroids and local irradiation was undertaken and fever, leukopenia and elevated serum creatinine levels returned to normal. Biopsies of the renal grafts of seven other patients with concurrent infection and elevated serum creatinine were all consistent with rejection, and immunofluorescent studies revealed no evidence of virus-complex nephritis.[27] Culture of 7 of the 8 biopsies revealed no evidence of viral infestation of the kidney itself.

Of the 37 patients whose onset of CMV infection could be determined to be associated with rejection episodes, 35 were given anti-rejection therapy consisting of graft irradiation and steroids.[7] Two kidneys regained normal function without treatment and four kidneys were lost despite treatment. These latter four kidneys demonstrated changes perfectly compatible with rejection.

Two patients were not treated for the elevated serum creatinine. When serum antiviral antibody reached a high titer, serum creatinine and temperature spontaneously returned to normal levels and remained there.[30] No renal biopsy was performed in these patients.

Forty-three additional patients had laboratory evidence of viral infections but the precise time of onset of the infection could not be determined since seroconversion had already occurred. Of these 43 patients, 18 (42%) had fever, 14 (33%) had leukopenia and 25 (58%) had rejection episodes associated with the diagnosis. It is impossible to determine whether the lower incidences of these clinical findings are related to the effect of pre-immunization against CMV or later diagnosis but it is clear that CMV infections were frequently associated with fever, leukopenia and clinical rejection episodes. Three of these 25 patients with rejection episodes have lost their kidneys by rejection.

Cytomegalovirus (CMV) infections occurred in the absence of other obvious complications in 89 patients. Six of these 89 patients died within one month of the diagnosis and none of the other 83 patients died within three months of the infection. Thus, 83 of the 89 patients recovered. The fever appeared self-limited in these 83 patients and usually abated after several days—to several weeks associated with the development of high complement fixing (CF) antibodies against the CMV virus. The leukopenia was usually short-lived and the white blood count reversed to normal after cessation of azathioprine (Fig. 2). Patients who died had persistent fevers (Fig. 4) and prolonged or repeated episodes of leukopenia despite cessation of azathioprine.

A total of 7 kidneys were lost by rejection within three months of the initial rejection episode associated

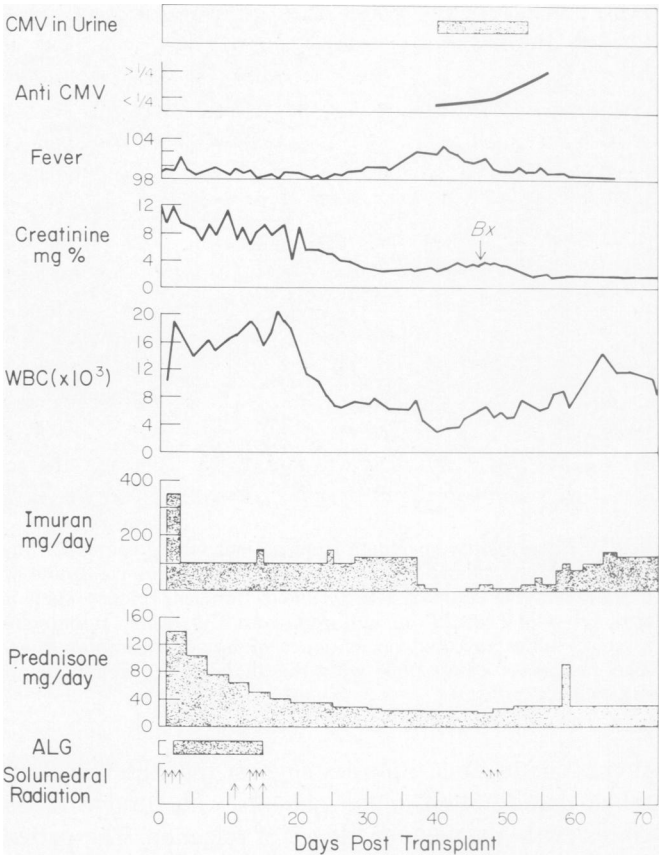

FIG. 2. Clinical course of a patient with typical CMV associated rejection reaction. Daily spiking fevers in the 5th posttransplant week were followed by leukopenia and elevated serum creatinine levels. Renal biopsy (Bx) demonstrated a typical acute rejection reaction (Fig. 3). Leukopenia abated when azathioprine was stopped, and the repection was reversed by increased steroids. The fever resolved as antibody to CMV developed. CMV was isolated from the urine.

with CMV diagnosis. All these 7 patients survived and were returned to dialysis. All 7 patients developed high antibody titers to CMV.

Of the 43 patients who had laboratory evidence of CMV infections when first studied and who had seroconverted, none died within three months after the CMV diagnosis was made. In contrast, of the 46 patients whose onset of infection was detected before antibody titers to CMV were detectable, 6 died—all within one month of the diagnosis of the infection by viral isolation. Only one of these 6 dead patients demonstrated a fourfold increase in antibody titers to CMV. The single seroconverter had a titer of 64 on the day prior to death. Histological examination of the kidneys of these six patients, revealed no evidence of rejection despite the fact that renal functional deterioration had occurred in the month prior to death and all patients had required hemodialysis.

Thus, two clinical syndromes seem to be associated with CMV; fever, leukopenia, renal functional deteriora-

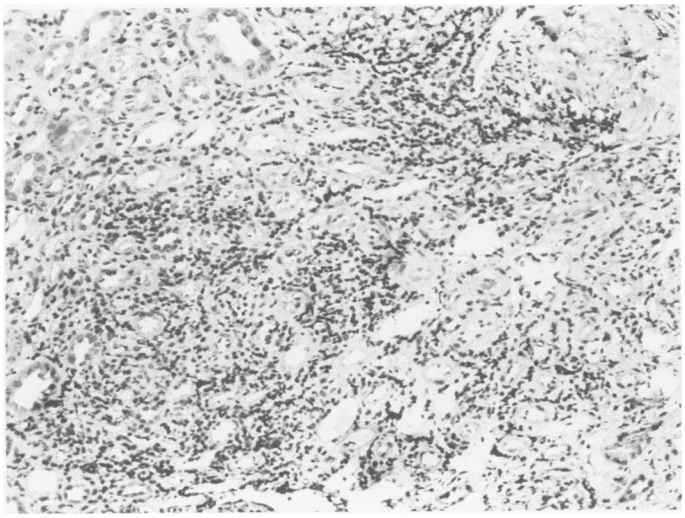

FIG. 3. Renal biopsy specimen from patient whose course is shown in Fig. 2. The biopsy was taken when the serum creatinine was elevated. CMV could not be isolated from the biopsy specimen although CMV could be cultured from the urine. Immunofluorescent studies revealed no evidence of immune complex disease. The picture is compatible with the diagnosis of renal allograft rejection.

tion occur in each. Biopsies suggest that the renal functional deterioration in the patients who survive is associated with histologic evidence of rejection. The patients who survive develop high antibody titers against CMV. The patients who go on to die, have normal kidneys despite clinical evidence of renal malfunction and do not develop antibodies to CMV.

The course of a typical lethal CMV infection is shown in Fig. 4. This lethal viral infection characteristically begins with spiking fever associated with mild generalized malaise; during this prodromal period bacterial and fungal cultures of blood, urine and cerebral spinal fluid are negative and no cause for the febrile episode can be identified. Prostration and orthostatic hypotention usually force the patient to be on permanent bedrest during the next 6 to 7 days. Lymphopenia, thrombocytopenia and mild deterioration of renal function are present. Mild arterial hypoxemia (pO_2 75 mmHg), not usually associated with radiological evidence of lung pathology, is also usually present from the onset of the febrile illness.

In the second week of the syndrome, new symptoms such as anorexia, nausea, vomiting, constipation, and abdominal pains appear. During this period, severe muscle wasting, peripheral edema, and lethargy are characteristic. A more severe arterial hypoxemia (pO_2 40–60mmHg at room air) is present. At this time, microscopic analysis of bronchial washing obtained at bronchoscopy may demonstrate the virus; still there is no other clinical evidence of lung pathology. The thrombocytopenia and lymphopenia persist, and, in 50% of the cases, leukopenia less than 5000 is present. Concurrently, laboratory evidence of moderate liver dysfunction and further deterioration of renal function appears. Except for the possible isolation of the CMV in biopsy specimen (kidney, bone marrow) bacterial and fungal cultures continue to be negative despite persistent high spiking fevers. The fevers are difficult to control by external cooling or anti-pyretics.

In the third week, there is a further deterioration of the patient's general condition. Muscle wasting is now more evident, peripheral edema is more marked, and icterus may be clearly evident. The severe hypoxemia, now requiring continuous O_2 therapy, is now associated with X-ray evidence of interstitial pneumonitis in about half of the cases. Liver and renal function further deteriorate (bilirubin >3mg%, SGOT, SGPT elevation in 75% of the cases and hemodialysis required in 75% of the cases). Hyperamylasemia associated with abdominal distention, decreased bowel sounds, diffuse rebound and tenderness is usually present in 50% of the

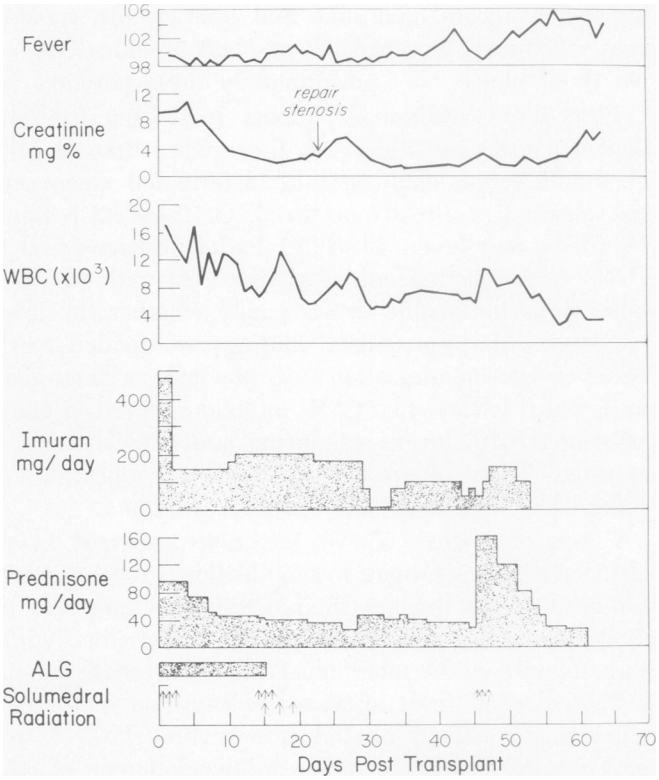

FIG. 4. Clinical course of a patient with CMV infection leading to death. Hypertension and renal malfunction was treated in the third posttransplant week with steroids and graft irradiation. Subsequently renal artery stenosis was repaired. Spiking fevers in the 6th posttransplant week was followed by renal malfunction and ultimately leukopenia. The fevers persisted until death. Bacterial and fungal cultures were repeatedly negative until the week prior to death. No antibodies to CMV were found. At autopsy there was no evidence of allograft rejection in the transplanted kidney.

patients. In these cases, diagnostic peritoneal lavage is sterile.

Characteristically, it is during this period that the majority of the patients (75%) presents evidence of CMV infection in cultures of sputum, urine, bone marrow and kidney biopsy; bacterial cultures negative until the third week now become positive for members of the intestinal flora.

In the terminal phase, lasting usually 5–8 days, there is further progression of the symptomatology. The patient is now semicomatose, with rapid progression to deep coma. High fever, arterial hypotention, labored respiration, tachycardia disproportionate to the degree of fever, and emaciation are present.

Periods of apnea develop early during this period requiring constant assisted mechanical ventillation, Icterus, petecchiae, ecchimosis, GI bleeding are a reflection of the further deterioration of the hepatic function. Hypofibrinogenemia, prolongation of PT, PTT, severe thrombocytopenia, uremia, all contribute to the hemorrhagic manifestation. The severe leukopenia (less than 2000mm^3) still associated with lymphopenia contribute to the terminal bacterial and fungal invasion. Irreversible pulmonary edema represents the terminal event.

At autopsy severe malnutrition, edema, petichiae and loss of subcutaneous fat are always present. Pneumonitis and pulmonary edema are universally present along with CMV inclusion bodies in the lungs. CMV can be cultured from multiple tissues, as can aspergillus fumigatis and a variety of gram negative organisms. Bile stasis, congestion, plasma infiltration and fatty metamorphosis as well as CMV inclusion bodies are found in the liver. The GI tract shows evidence of diffuse ulcerations. The kidneys show no evidence of acute rejection though CMV inclusion bodies can be found and CMV cultured.

Immunologic responses of patients who recovered from the virus infections differ markedly from those patients who died. The micro-PHA stimulation responses of 61 patients stabilized on maintenance doses of azathioprine and prednisone had mean responses of 1436 ± SD 1708 CPM. This mean response is greater than two standard errors below the mean of the normal ($p < .0001$). The patients who had recovered from viral infections had a mean response of 1556 CPM which is not significantly different from the entire patient group or a group of eight patients who never developed virus infections, (1547 CPM). In addition, significant changes in the micro-PHA responses, attributable to the CMV infections, were not detected during the course of those infections.

In contrast, the mean micro-PHA response of four carefully studied patients who died of CMV infections was less than 400 CPM throughout their course. They showed no increase in PHA responses when immunosuppression was reduced or even stopped. In addition, their kidneys showed no evidence of renal allograft rejection at postmortem examination despite long periods off the immunosuppression prior to death. Low micro-PHA responses, however, could not always be correlated with increased susceptibility to severe infection or failure to reject renal allografts. Thus, it was not unusual for patients whose clinical courses were free of serious clinical infection to have periods of weeks with equally low responses. In this latter group, however, micro-PHA responses eventually rose to levels equalling or exceeding mean responses for patients on maintenance doses of immunosuppressive therapy. Also, rejection episodes occurred in these patients despite moderately low micro-PHA responses.

The macro-PHA test tests the function of washed lymphocytes in the absence of blood. The average response of the immunosuppressed renal transplant patients on maintenance therapy was 50,283 CPM, while the average response during the first two weeks after transplantation was 46,895 CPM (normal 48,456). These responses can therefore be considered normal. One of the four patients who died and who demonstrated a micro-PHA response of only 95 CPM four days before death, had a macro-PHA response of 45,478 CPM on that same day.

Complement fixing (CF) antibody responses to CMV of the surviving patients were usually ten to 100 times higher than those found in non-immunosuppressed patients. Others have reported[48] that normal patients infected with CMV respond with titers of 1:16 to 1:64. We have observed rises in antibody titer of this same magnitude in non-immunosuppressed patients infected with CMV. Of the immunosuppressed patients who produced CF antibody to CMV, 95% had antibody titers of 1:64 or greater. The patients who died did not respond with CF antibody to their CMV infection throughout their long clinical course.

Discussion

Cytomegalovirus (CMV) infections have been frequently observed in patients receiving organ transplants since the Denver group first described this phenomenon in 1964.[17] Hill *et al.* discussed 15 patients with evidence of CMV infection at autopsy out of a total of 32 patients who had died after receiving transplants.[7] Evidence of infection was found in the lungs as well as other organs. These observations have been repeatedly confirmed.[2,3,8,11,12,16,30,41,42,50] CMV in transplant patients can be most easily recovered from the urine but frequently also from the sputum or from bronchoscopy specimens. In our experience, viremia, even utilizing cultures of buffy coat, has been difficult

to detect. The fact that antibodies to CMV appear 3–4 weeks after a febrile illness but only a few days after viral isolation strongly suggest that viremia occurs but is not being detected by our current techniques. In any case, we can confirm the fact that CMV infections occur in 70–90% of renal transplant patients.

Laboratory evidence of CMV infections is so common in asymptomatic transplant recipients, that it has been regarded as an incidental finding. However, our continuing epidemiological studies in this patient population demonstrate that the first appearance of CMV is almost always accompanied by a clinical illness.[29,30] The most characteristic and frequent picture of CMV infection seems to be the appearance several weeks posttransplant of fever, leukopenia, and signs of renal functional deterioration. The fever is the first sign, most often followed sequentially by leukopenia and renal malfunction. Biopsies of kidneys reveal typical features of rejection and the absence of immune complex disease and/or gross infection of the kidney itself. In fact, kidney biopsies failed to produce evidence of virus infection in almost all circumstances, even though the urine was the most common source of isolated virus. The rejection reaction responds to antirejection treatment, leukopenia responds to decreases in azathioprine dosage and the fever abates as antibodies to CMV develop. Recovery is the general rule though irreversible kidney rejection may appear.

Six of our patients died within one month after the laboratory diagnosis of CMV infection. The clinical characteristics of these patients do not differ during the early phase from patients who recover—fever, leukopenia and renal functional deterioration are also present. Certain differences can be detected, however. The six patients had little histological evidence of rejection within the malfunctioning kidney, antibody to CMV did not usually appear, and the lymphocyte responses to mitogen, though intact, were suppressed in the presence of whole blood.[31] In short, evidence of immunologic reactivity was lacking. This difference in immunologic responsiveness between patients who recover and those who die when infected with CMV is in harmony with findings that CMV itself is a profoundly immunosuppressive virus.[20,21] Howard *et al.* found that CMV-infected mice could not reject skin grafts or respond to sheep red blood cells (SRBC) until recovery had occurred. The patient already immunosuppressed with antilymphoblast globulin, azathioprine, prednisone, radiation, and methylprednisolone appears to be even more profoundly depressed by CMV infections. Such patients appear susceptible to superinfection with gram negative bacteria and other opportunistic organisms which sequentially infect and ultimately kill the patient. At autopsy a "mixed" infection is frequently found[45] but evidence of organisms other than CMV is usually lacking until the preterminal phase of illness when blood and sputum cultures finally became positive for bacteria.

There are a number of problems unresolved by these studies. The first is the source of CMV: A number of epidemiologic studies have suggested that CMV infections are common in early neonatal life and that most patients will have evidence of antibodies to virus by adult life.[26] The virus, however, appears to remain within the body in latent form, and can be reactivated despite the presence of high complement fixing or neutralizing antibody titers.[51] Alternatively, CMV infections in transplant patients may be acquired from the environment, transfusions,[39] or even the donor organ itself.

It is, however, generally difficult to culture CMV from bank blood.[34] The virus, when present in blood, is most easily found in the leukocytes and transplant patients are given only leukocyte poor or frozen blood. Similarly, cultures of donor kidneys have not revealed virus. In short, reactivation of latent endogenous virus is the most likely route of infection.

Studies are currently under way to strain-type CMV isolates from our renal allograft patients and to determine differential neutralizing and complement-fixing titers to the various strains isolated. This should enable us to determine whether sequential CMV infections are acquired from exogenous sources, or result from reactivation of endogenous, latent virus.

We have previously discussed the complex interrelationships between reactivation of latent viral infections, immunosuppression, and allograft rejection.[30] On one hand, it is possible that a rejection episode might activate a latent virus infection.[30] Activation of a latent herpesvirus infection has been demonstrated in rabbits[14] in conjunction with anaphylactic shock, or a second exposure to the antigen. Graft versus host reactions have also been shown to activate latent viruses.[4,18,19] Other studies have shown that lymphocytes, transformed by mitogens or antigens, can be more easily infected than can normal lymphocytes, and such cells appear to support viral multiplication much better than do nontransformed cells.[24] It seems likely that lymphocyte transformation is an integral part of the rejection process; thus, recently transformed lymphocytes would be available for virus infection. Therefore, clinical or subclinical, rejection occurring soon after transplantation might activate a latent CMV infection.

The second possibility is that virus may act as a nonspecific stimulus or adjuvant upsetting the very delicate immunologic balance between donor organ and host. The postulate presupposes that there is a state of subclinical rejection present in most allografted kidneys

and that a state of relative immunologic adaptation of host to graft becomes established with time. Random biopsy specimens of well tolerated allografts usually show some evidence of rejection, however minimal.[13,38,43] In addition, humoral and cell-mediated immunity to functioning allografts have been repeatedly demonstrated.[13,33,36,37,44] Similarly, there is much evidence to support the idea that bacterial agents (i.e. endotoxin, BCG, infections) can nonspecifically stimulate immunologic processes.[52] Endotoxin, for example, can facilitate the rejection process itself.[1] Perhaps viruses, too, or virus antibody interactions can do likewise. This possibility receives further support from patients with postperfusion mononucleosis caused by CMV. In these patients delayed hypersensitivity to ampicillin and other immunologic abnormalities developed concurrent with their virus infection.[22,25] Furthermore, if we can compare this clinical syndrome to postperfusion mononucleosis, "exposure" to infectious doses of virus precedes clinical rejection by about 2 to 4 weeks. Although one cannot say that infection triggers rejection just because it precedes it, these data lend added weight to this possibility. Further weight in support of this hypothesis derives from the fact that lethal CMV infections are not associated with histological evidence of rejection despite renal malfunction. In these patients, it is unlikely that rejection reactivates the virus, but that the virus is reactivated by immunosuppressive drugs.

In sum, two paradoxical responses to the virus are possible. The relatively immunocompetent transplant recipient behaves as though the virus were an immunologic adjuvant triggering an allograft rejection. The immuno*in*competent patient may be further immunosuppressed by the virus and develop sequential opportunistic infections and die. These paradoxical responses to CMV virus have been reproduced by Howard *et al.* in the murine CMV model (Howard, unpublished observations). A primary CMV infection in a mouse is immunosuppressive, and the mouse cannot reject skin allografts or make antibody to SRBCs. Mice who are immune to CMV, have accelerated responses to primary allografts and better antibody responses to SRBC if primary antigenic challenge is accompanied by a second CMV inoculation. If these results are applicable to the clinical situation, we might expect that CMV infections were primary infections in those patients who died, and were secondary or reactivation of latent virus in the patients who survived.

The profoundly immunosuppressed state of patients with potentially lethal infections, and the absence of histological rejection reactions should permit the rational administration or withholding of antirejection therapy in these cases. Within a few days after development of fever and renal malfunction, PHA responses in autochthonous plasma and renal biopsy can be performed. If renal allograft rejection is shown in the biopsy and PHA responses are normal, one might assume that the self-limited viral syndrome is present. On the other hand, if a typical viral infection is accompanied by renal malfunction but a normal kidney biopsy and PHA responses are depressed, immunosuppression might be stopped and the patient allowed to recover.

The causes of renal malfunction in the lethally infected patient are not totally clear. These patients appear to be hypotensive, vasodilated and edematous. In this state of chronic viremic shock, renal perfusion may be inadequate to maintain adequate function. Alternatively, the kidney could be infected by the virus itself which shows a propensity for epithelial tissues. Bone *et al.*[6] have recently reported that kidney biopsies in febrile leukopenic CMV infected transplant patients showed tubular cell degeneration and intracytoplasmic viral bodies.[6]

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